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COLLEGE OF AGRICULTURE UNIVERSITY OF NEBRASKA
AGRICULTURAL EXPERIMENT STATION
RESEARCH BULLETIN 70

Cephalosporium Wilt and Die-back of the White Elm

R. W. GOSS AND PAUL RAYMOND FRINK
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SUMMARY

1. A new disease of the white elm has been found to be common in eastern Nebraska.

2. The causal organism has been isolated and its pathogenicity proved by inoculation.

3. The fungus belongs to the genus Cephalosporium and is considered to be the same as that described by Curtis May of Ohio and found by him in specimens obtained in Iowa, Missouri, New York, and Washington, D. C.

4. The morphology and cultural characters of the organism are described.

5. A survey of the elms in street plantings in Lincoln revealed about 1.5 per cent of the trees showing typical symptoms of the disease and an additional 24.2 per cent showing slight symptoms that may have been due to this disease. A total of about 30 per cent of the trees examined were diseased or dead or had been replaced.

6. The symptoms occur as a slight drooping of the leaves with a yellowish green mottling, followed by an upward rolling and browning. Eventually the twig or branch appears as a definite die-back. Internally a brown, diffuse streaking of the vascular tissue occurs that usually accompanies and precedes the external symptoms. When the trunk is infected the leaves are smaller, yellowish, and rolled in the spring and the entire top may develop a rapid wilt.

7. The disease was produced in white elm seedlings by inoculation of either roots, trunk, or twigs. The symptoms appeared as early as 11 days later and death sometimes occurred within three months. The rapidity of progress of the organism through the tree depends upon the point of infection. The disease advances more rapidly upward than toward the roots.

8. It is probable that because of the location of the fungus within the tree, insects play an important part in its dissemination.

9. No infection was obtained by inoculation of Chinese elms.

10. Suggested methods of control involve careful inspection and removal of infected trees in the nursery and the removal of either the entire tree or affected portions of trees in ornamental plantings.

11. Further work is needed, such as studies of varietal susceptibility and the possibility of transmission by soil and insects.

Cephalosporium Wilt and Die-back of the White Elm

R. W. GOSS AND PAUL RAYMOND FRINK

During the spring of 1931 the attention of the senior author was called to the sudden death of a number of large white elm trees (*Ulmus americana* L.) at Crete, Nebraska. Some of the symptoms of the disease appeared similar to the published descriptions of the Dutch Elm disease caused by *Graphium ulmi* Swartz. Specimens were collected and sent to the Dutch Elm Disease Laboratory in Ohio. Similar specimens were cultured at Lincoln, and specimens were obtained and cultures made from a tree on the campus of the Nebraska Agricultural College. This tree appeared to be affected with a similar trouble.

Mr. Curtis May reported from the Dutch Elm Disease Laboratory that this was not the Dutch elm disease [1] but that his isolations yielded an organism which he tentatively placed in the genus Cephalosporium Corda. In October, 1931, May [2] published a short note reporting the isolation of this organism from specimens obtained from Iowa, Missouri, New York, and Washington, D. C., and stated that inoculation tests had proved its pathogenicity. He also gave a brief description of the organism and the symptoms of the disease. Organisms referred to the same genus were reported by Liming [3] as having been obtained from 336 of 1,407 trees from which isolations were made at the Dutch Elm Disease Laboratory.

Mr. May kindly forwarded a culture of his organism for comparison with the isolations made in Nebraska and the organisms were found to be similar with only a few minor differences in culture. His cultures and those isolated in Nebraska produced similar symptoms when inoculated into elm seedlings. The Nebraska disease was therefore considered identical with that described by May.

A preliminary survey of the elms in Lincoln was undertaken and investigations were started to determine the extent and severity of the disease and if possible the means of spread and methods to be employed for its control. Owing to the necessity of discontinuing this investigation, the results of the work up to the present time are here reported.

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[1] The Dutch elm disease was first discovered in the United States in 1930 and has not been found in Nebraska. At present the disease has been found in Ohio, New York, New Jersey, Connecticut, and Maryland, and all infected trees are being eradicated. Information regarding this disease is available in a publication by Curtis May and G. F. Gravatt, *The Dutch Elm Disease*, U. S. D. A. Circ. 170, 10 pp., 1931.

[2] Curtis May, "A New Elm Disease," *Science*, n. s. 74:437, 1931.

[3] O. N. Liming, "Elm Diseases in America," (Abst.), *Phytopathology*, 23:21, 1933.

DISTRIBUTION AND PREVALENCE

In the fall of 1932 a survey was made of the elm trees, chiefly in street plantings, in various parts of Lincoln. A total of 3,289 trees were examined, of which 85 per cent were 6 inches or more in diameter. Trees showing severe symptoms corresponding to those obtained by inoculations with Cephalosporium totaled 1.5 per cent, of which 1.1 per cent were large trees. In addition 24.2 per cent of the trees showed slight symptoms which may or may not have been due to Cephalosporium, while 0.8 per cent were dead and 1.3 per cent were affected by injuries or diseases definitely due to causes other than Cephalosporium. There were also 1.9 per cent that were replants. About 30 per cent, therefore, were diseased or dead or had been replaced.

Of those listed above as being severely infected there were 29 trees in which the symptoms occurred throughout the tree, indicating a rapid systemic infection possibly originating in the roots, as contrasted with the usual progressive development of the disease starting with individual branches. Isolations were made from nine of these trees, but Cephalosporium was obtained from only one. Cephalosporium was isolated more easily from trees with characteristic die-back symptoms; such trees usually yielded pure cultures of Cephalosporium in 60 per cent or more of the isolations.

In 1933, isolations were made from trees collected from 10 nurseries scattered through central and eastern Nebraska. Cephalosporium was obtained from four of these collections. The organism has also been isolated from other specimens sent in during the past two years.

Isolations from seven Chinese elms (*U. pumila* L.), showing variable symptoms of die-back or wilting, failed to reveal the presence of Cephalosporium.

The organism has thus been repeatedly isolated from white elms of all sizes from seedlings to trees 24 inches in diameter, obtained in various parts of Nebraska. Hence the disease has a fairly wide distribution in this state and is quite possibly the cause of many of the numerous reports of the death of elms. The addition of this state to those already reported by May indicates that the disease is quite general throughout the central and eastern portions of the United States.

Verrall [4] in 1930 reported a disease of elm occurring in Minnesota with somewhat similar symptoms, but the organism he isolated produced pycnidia in culture and he referred it

[4] A. F. Verrall, "Die-back of Elm in Minnesota," *Phytopathology*, 20:1,004-1,005, 1930.

tentatively to Cytosporella. The cultures of Cephalosporium isolated in Nebraska have never produced a pycnidial stage, but it is possible that such a stage might occur upon other media.

SYMPTOMS

Branches of trees inoculated with pure cultures of Cephalosporium and branches naturally infected develop similar symptoms, while root inoculations present a very different picture of the disease.

FIG. 1.—The first symptoms of wilt resulting from inoculation of a white-elm seedling by the introduction of Cephalosporium into the trunk. An irregular yellowish-green mottling occurs along the midrib and veins, followed by a rolling, wilting, and browning of the leaves and a drooping of the twig.

Infection of Branches.—The first external evidence of the disease is a slight drooping of the leaves, accompanied by a yellowish-green mottling which begins at the base of the leaf and gradually spreads up along the midrib and then out along the veins to the margins (Fig. 1). This is followed by the browning of the tissues and the death of the leaves at the margins, accompanied by an upward rolling. There is also a drooping of the affected twig and the brown, rolled leaves often persist for a month or more.

When only a few vessels on one side of the tree or branch are infected the symptoms develop only on those branches which have tracheary tubes leading out of the infected vessels (Fig. 2 A). When the whole vascular ring is infected, rapid wilting and death occur in all parts above the infected portion (Fig. 2 B). The disease progresses rapidly above the point of infection but very slowly below. Frequently trees that are severely infected will send out shoots below the point of infection.

A B

FIG. 2.—White-elm seedlings inoculated just below the first branch. (A) Showing the development of the disease limited to one side of the tree 40 days after inoculation. (B) Showing the result of invasion of the entire vascular system with the production of healthy shoots just above the ground level 52 days after inoculation.

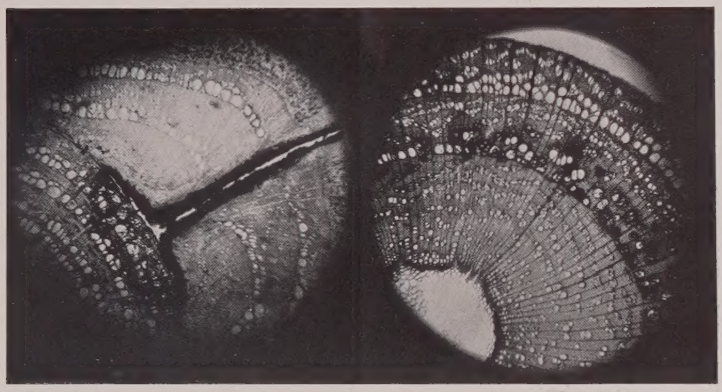
Internally the disease is manifested by a brown, diffuse streaking of the vascular tissues of the current season's growth (Fig. 3). This internal discoloration is evident in advance of the external symptoms.

FIG. 3.—A section of a diseased branch showing the brown, diffuse discoloration of the sap wood.

May describes the internal discoloration as being more diffuse than that occurring with either the Dutch elm disease or Verticillium wilt. He also describes an external blackening of the bark above the point of infection. This symptom has not been noted in these investigations.

Observations of naturally infected trees indicate that two to four years are usually required to kill a good-sized tree (Fig. 14, A and B), while inoculated seedlings are often killed during the current year. Severely infected trees were usually badly infested with borers and it is possible that insects may

play a part in transmitting the disease, as the discoloration of the vascular tissue often appeared to originate at such insect punctures and the organism could be isolated from diseased tissue such as that shown in Figure 4 A.



A B

FIG. 4.—(A) Cross section (x19) of a twig showing an insect puncture with the discoloration of the vascular tissues from which Cephalosporium was isolated. (B) (x19) Cross section of a diseased twig with the discolored vessels either wholly or partly plugged.

Root Infection.—Less evidence is at hand concerning the infection of trees through the roots. When the roots of seedlings are inoculated early in the spring the development of the disease is very rapid. The foliage is scanty, the leaves are small, yellowish, and considerably rolled, and the death of the tree often occurs during the current season.

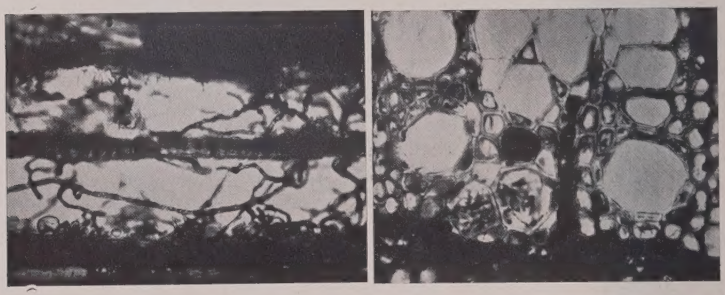
The organism has not been isolated from the roots of naturally infected trees, but numerous instances have been recorded of large trees dying in one year with symptoms corresponding to those obtained by root inoculation, and the organism has been isolated in one instance from the lower branches of such a tree. The stumps of these trees and also of large trees that have died following progressive wilt and die-back do not send out sprouts, thus indicating the death of the roots. Much more work needs to be done on this phase of the disease, as the evidence collected to date is fragmentary and incomplete.

PATHOLOGICAL ANATOMY

The gross characteristics of infected tissues have been briefly described in the previous section. The brownish dis-

coloration of the vascular tissue is diffuse and uniform, except in the earliest stages when it appears as apparently discontinuous brown spots.

Portions of twigs showing discolorations were fixed in formal-acetic alcohol and sectioned with a hand microtome. Two staining methods were used.[5] Hematoxylin-erythrosin stain was very satisfactory for bringing out the mycelium without staining the host tissue too deeply. The gold-chloride method was found satisfactory for photographing low magnifications, as the infected tissues stained from deep purple to black with the healthy tissues staining pink.



A B

FIG. 5.—(A) Longitudinal section (x246) of infected vessels showing mycelium and tylose-like bodies. (B) Cross section (x255) of a petiole of an elm leaf just starting to show wilt symptoms. Two of the vessels contain mycelium and a third is plugged with a yellow gum-like substance.

Examination of stained sections showed the vessels to be filled with mycelium usually accompanied by tyloses or tylose-like bodies which were also present in adjacent vessels not containing mycelium (Fig. 5 A). The mycelium was confined to the vessels, and eventually they become completely plugged, the contents appearing in unstained sections as a yellow gum-like substance (Fig. 4 B).

The mycelium is not only present in the discolored bundles but also for several inches in advance of the discoloration (Fig. 13, p. 21). It is present in the leaf petioles of infected twigs as shown in Figure 5 B. The cells adjoining the vessels containing mycelium die and show the dark purple or black

[5] Sections were placed in 95 per cent alcohol for 5 minutes, washed in water, and stained with Delafield's hematoxylin for 20 minutes, destained in a weak hydrochloric acid solution, washed in water, and counter-stained 2 minutes in erythrosin (3 per cent in aqueous phenol). The excess stain was removed in water and the sections quickly dehydrated in 95 and 100 per cent alcohol, cleared in xylol, and mounted in balsam. The other method used was as follows: Sections were placed in a 1-to-1,000 solution of gold chloride for 12 hours followed by 30 minutes in phloxine (in 80 per cent alcohol), dehydrated, cleared in xylol, and mounted in balsam.

stain with gold chloride, even though they do not contain mycelium. The organism has not been observed in any tissues other than the vessels and is apparently spread radially only by the anastomosing of the tubes.

The large masses of mycelium present in the tubes, accompanied by the tylose-like bodies and the eventual plugging of such tubes, with the death of the adjacent cells, would account for the resulting wilting of all portions of the tree supplied by these tubes without the production of any toxic principle. The disease is therefore considered a vascular mycosis.

THE CAUSAL ORGANISM

Taxonomy and Morphology.—The organism isolated and described by May as the cause of a similar disease was tentatively classified by him as a Cephalosporium. The authors are in accord with May in placing this organism in the genus Cephalosporium rather than in any of the closely related genera. We have been unable in an extensive search of the literature to find any published description of a species in the genus Cephalosporium or in any of the closely related genera that satisfactorily fits the characters of this organism and it should probably be considered as a new species.

The organism studied in this investigation is characterized, on potato dextrose agar, by a well-developed, hyaline, septate mycelium moderately branched at an angle of about 70 degrees. The simple, usually unbranched, conidiophores are not apically swollen and vary in length from 7 to 18 microns. They are from 0 to 3 or 4 septate and are usually shorter and of smaller diameter than non-fruiting hyphae (Figs. 6 and 7). Usually they are found in the older portions of the culture. The conidia, borne successively at the apex of the conidiophores, are small, elliptical, hyaline, one-celled spores averaging 1.9 x 4.5 microns (average of 200 measurements). The successive conidia are held in place by a mucilaginous substance in which 50 to 80 spores may be massed into a globular head.

The spore heads are formed after 6 to 8 days' growth, as shown in Figure 7. The process of spore development can be followed by saturating the atmosphere and thus dispersing the spore head.

Gross Cultural Characters.—*Potato dextrose agar:* The fungus forms a slow-growing, circular, raised, cottony colony, white when young but turning brown [6] with age, especially at the surface of the medium, which is always darker than the aerial hyphae. The hyphae at the margins of the colony are

[6] The shade of brown on this medium, according to Ridgeway's Color Standards, would range from light buff to cinnamon brown.

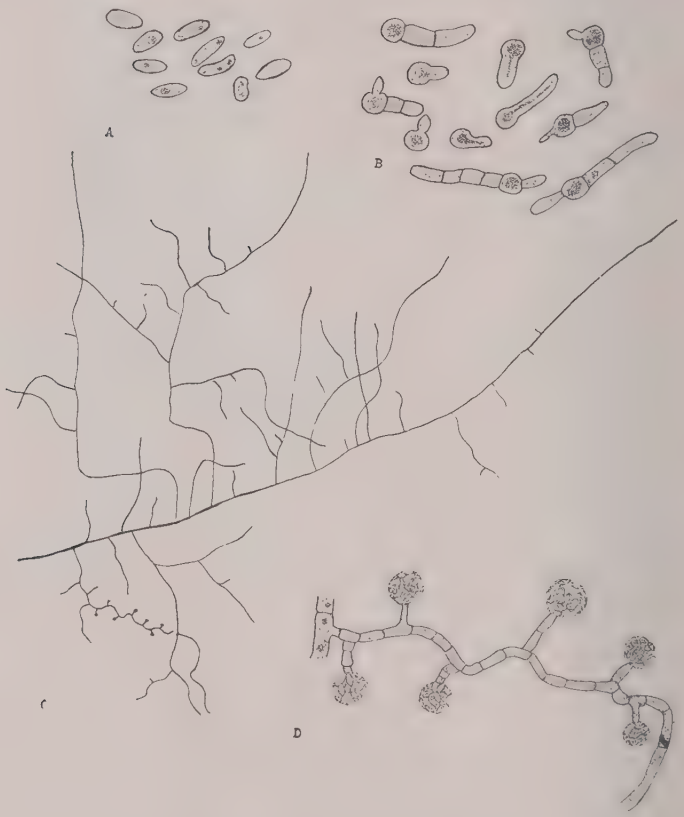


FIG. 6.—(A) Cephalosporium spores, x1130. (B) Spores germinating on potato dextrose agar, x1130. (C) Type of marginal growth in petri-dish cultures on potato dextrose agar x20. (D) Fruiting branch of (C) enlarged to show its structure, x220.

long, slender, and unbranched. While growth on this medium is described as slow, it is more rapid than on other agars tested, producing a colony 7 to 8 cm. in diameter in two weeks. *Nutrient agar:* Growth is almost equal to that on potato dextrose agar for the first three days and about half as much for the next five days, after which little increase occurs. Color characters are very similar to those on potato dextrose. *Potato plugs:* A slow-growing, raised, cottony growth is produced, dark brown at the surface of the medium with light-brown aerial hyphae. *Rice:* Growth moderate, brown at the surface of the medium, with scanty white aerial mycelium.

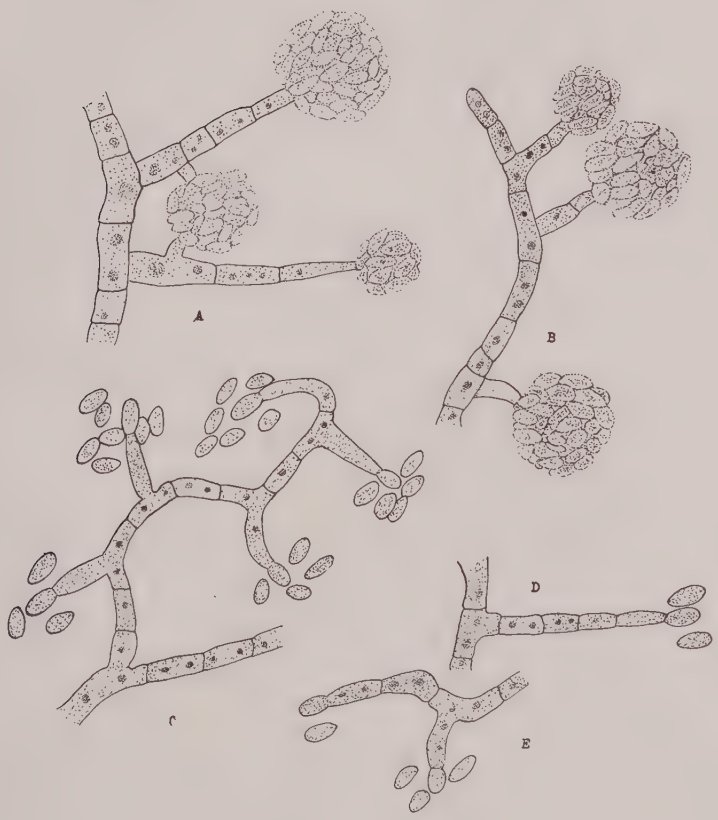


FIG. 7.—Spore formation in hanging drop cultures on potato dextrose agar at pH 7.0, 27° C. A and B show the variation in segmentation of the conidiophores and the masses of spores held in place by a mucilaginous substance. The spore heads are dispersed (C) by saturation of the atmosphere, which allows observation of conidial development (D and E). A and B, x980; C, D, and E, x1200.

Yellowish to dark-brown coloration of the medium. Abundant production of spores. *Elm leaves:* Growth comparatively rapid with considerable aerial mycelium. Sporulation moderate. Brown to reddish-brown color of substratum. Mycelium often occurring in rope-like strands. *Melilotus stems:* Growth rapid, aerial mycelium abundant, brown in color. Sporulation abundant but less so than in rice cultures. *Wheat straw:* Growth very slow, scant aerial mycelium, and very scant sporulation. *Potato broth:* A loose colony of mycelial growth

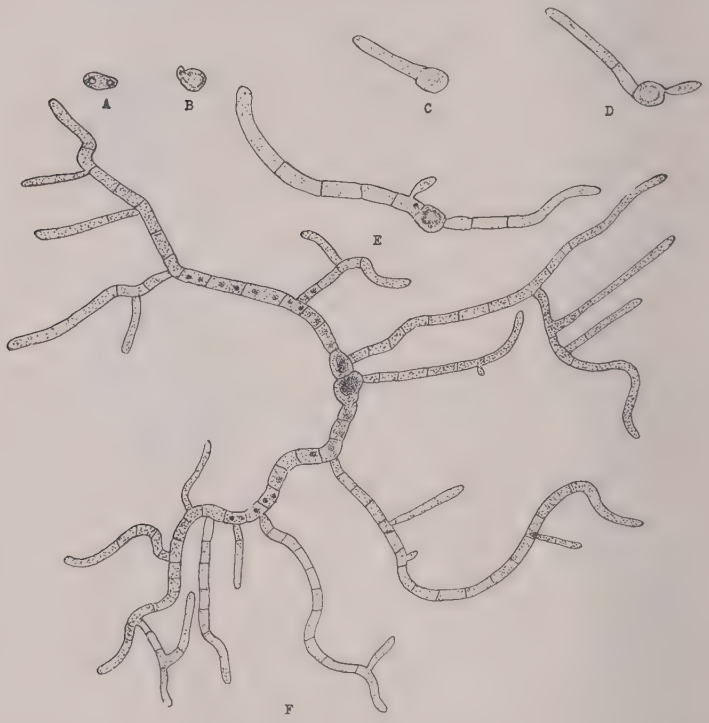


FIG. 8.—Germinating spore and developing mycelium on potato dextrose agar, at pH 7.0, 27° C., x1200. (B) 14 hours after planting on agar, (C) 18 hours, (D) 24 hours, (E) 30 hours, and (F) 40 hours.

is produced at the bottom of the tube. Small star-shaped colonies are attached to the wall of the tube, larger and more numerous near the surface of the medium, with a ring of such colonies developing at the surface. With the addition of dextrose the growth is heavier at the bottom of the tube, and, while a few small colonies develop at the surface, no colonies are attached to the walls. *Nutrient broth and Pfeffer's solution:* Growth is scant in both media, appearing as a small colony at the bottom of the tube.

In comparing the culture of Cephalosporium obtained from Ohio with the Nebraska isolations, it was found that while they were indistinguishable morphologically, there were slight differences in gross cultural characters. The Ohio culture always tended to produce a denser, heavier pad of mycelium on the surface of the medium and to mound up, while the Ne-

braska culture was looser and slightly more spreading. On potato dextrose agar the agar always turned a rose-red color after about one month's growth of the Nebraska cultures, while this did not occur with the Ohio culture.

Effect of Temperature on Growth.—The effect of temperature was studied by growing giant colonies in petri-dish cultures of potato dextrose and nutrient agar adjusted to a pH of 7.0. Freshly poured plates were inoculated at the center of the dish with a 3 mm. disc of a culture obtained from the young mycelium of a giant colony on the same agar. Four cultures were used for each medium at each temperature and the radial growth was measured daily for 14 days. Eight sets were run in incubators maintained at 3.5°, 10°, 15°, 20°, 25°, 30°, and 35° C.

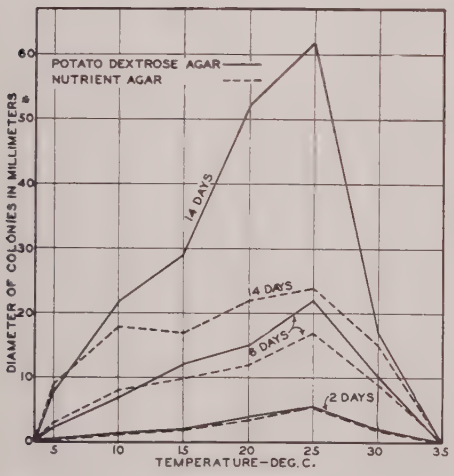


FIG. 9.—Graph of the growth temperature curve of Cephalosporium on potato dextrose and nutrient agar as determined by radial development.

Figure 9 shows the differences in growth at the various temperatures on both media at 2-, 6-, and 14-day intervals. The optimum temperature for growth on both media was 25°, while growth was not observed at the two extreme temperatures, 3.5° and 35° C. The data for the daily increments of growth are not presented but these data showed that at the optimum temperature the growth on the two media was about the same for the first three days, after which the growth on potato dextrose agar was about double that on the nutrient agar for the next five days, and that thereafter the daily increment continued about the same on potato dextrose but practically ceased on nutrient agar. This difference was not evident at the low and high temperatures, where the growth on nutrient agar was about equal to that on potato dextrose agar. In other words, the curve was flatter, probably because of the smaller effect of temperature on growth with an unfavorable medium.

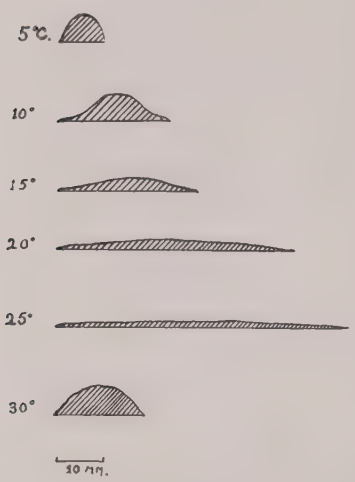


FIG. 10.–Sketches showing types of growth produced at different temperatures. Shown as cross sections of petri-dish cultures on potato dextrose agar after 14 days' growth at pH 7.

and potato dextrose agar adjusted to hydrogen-ion concentrations varying from pH 2.0 to 10.0 at 1.0 intervals. On potato dextrose agar the maximum growth occurred at pH 6.0 while on nutrient agar the maximum growth shifted from pH 6.0 at two days to 5.0 at six days and to 4.0 after ten days, where it nearly equaled the growth on potato dextrose agar at the end of the two weeks' period (Fig. 11). At pH 3.0 more growth

Such data as those presented above do not, however, indicate the effect of temperature upon the total growth of the organisms, as is evidenced by an examination of Figure 10. The thickness of the colony which was very noticeable at 5° C. decreased rapidly as the temperature approached the optimum and again thickened greatly at the maximum temperature.

Effect of Hydrogen-ion Concentration. — *Exp. 1.* — Cultures were made and relative growths determined as in the temperature tests on nutrient

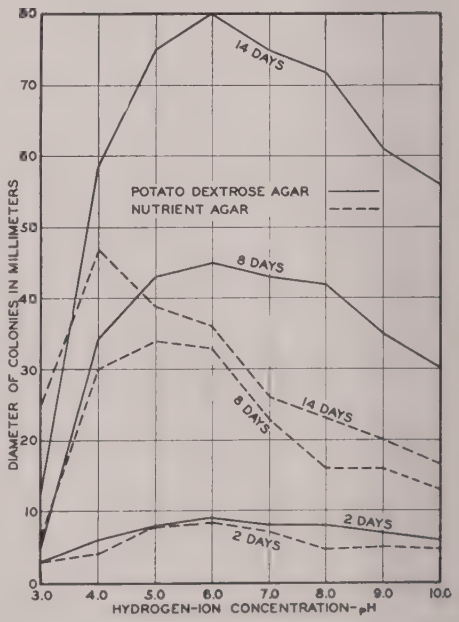


FIG. 11.—Graph showing the relation of initial H-ion concentration to the growth of Cephalosporium as determined by radial development on potato dextrose and nutrient agar.

occurred on nutrient than on potato dextrose agar. The growth at high pH's was much greater on the potato dextrose agar.

Exp. 2.—In an attempt to study further this difference in the action of the organism on the two media and to explain the apparent shifting of the optimum pH on the nutrient agar, another experiment was planned in which both the Nebraska and the Ohio cultures were used. Nutrient agar and potato dextrose agar were adjusted to a range of H-ion concentrations from pH 3.0 to 8.0 at 0.5 intervals. The experiment was divided into three sets of duplicate cultures with each organism on each of the two media.

Set 1 consisted of giant cultures in petri dishes as in the preceding experiment. Daily measurements were made of the diameter of the colonies to determine relative growths at the different H-ion concentrations.

Set 2 was similar to Set 1 except that indicator media were used to determine the daily change in H-ion concentration of the medium caused by the growth of the organism.

Set 3 consisted of slant cultures on media adjusted to the same H-ion concentrations as in Set 1. At the end of the experiment the agar was liquefied, the mat of mycelium removed, and the pH of the medium determined.

The latter set served as a check on Set 2, in which the indicators were found to influence the growth of the fungus. Methyl red inhibited growth, while brom-phenol blue, brom-cresol purple, and phenol red appeared to stimulate it slightly.

On nutrient agar the greatest growth during the first three days occurred at H-ion concentrations of 5.5 to 7.0. By the eighth day the maximum growth was found to be in the pH range of 4.0 to 5.0 and at the end of two weeks the growth was greatest in the range 3.5 to 4.5.

This shifting of the pH optimum for growth was apparently due to a change in the reaction of the medium caused by the growth of the fungus. The use of the indicator media in Set 2 showed a gradual change in the pH of the acid media toward neutrality. The determination of the final pH after two weeks' growth in Set 3 showed that the cultures originally adjusted to a pH of 3.0 had changed to 6.8, those at 3.5 to 7.2, and all others on the acid side had been changed to 7.4-7.6. The most alkaline medium, pH 8.0, had changed to 7.5. Both cultures of Cephalosporium reacted similarly on nutrient agar.

On potato dextrose agar the maximum daily growth throughout the test occurred over the pH range of 6.5 to 7.5 with both organisms. With this medium the shift in reaction was likewise toward neutrality but not nearly so rapid or ex-

tensive as in the nutrient agar. The Nebraska culture did not change the reaction more than 0.9, and the change was toward a final pH of 6.4 for all tubes originally at pH 5.5 to 7.0. The Ohio culture changed the acid media very little but did reduce the pH in all tubes originally above pH 5.5, the tubes at 7.0 to 8.0 being changed to 6.3.

Effect of Ultra-violet light.—The effect of ultra-violet light upon growth and sporulation was tested by exposing giant colonies in petri dishes with cellophane covers to a 450-watt mercury-vapor bulb (General Electric sun-lamp) at a distance of 50 cm. for periods of 1, 5, 15, 30, and 60 minutes on three consecutive days. The longer exposures inhibited the growth of the colonies and caused them to take on a darker brown color. The fungus was not killed at any of the exposures, and sporulation was not materially altered.

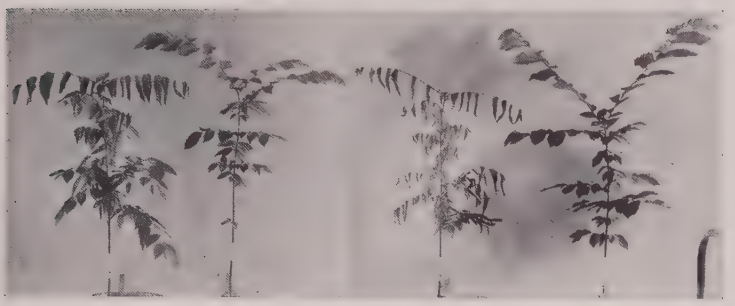
INOCULATION EXPERIMENTS

Experiment I — Preliminary Pathogenicity Tests. — In these inoculation tests white elm seedlings about three feet in height were grown in the greenhouse and inoculated after the foliage was well developed. They were inoculated by inserting mycelium into incisions made just below the first branch and covering with moist cotton held in place with gauze.

A number of the organisms that had been most consistently isolated from diseased elms were used in these inoculations, together with the Nebraska and Ohio cultures of Cephalosporium. The Cephalosporium cultures were the only ones that caused infection and the results were similar with both strains of the organism. The first symptoms appeared 11 days after inoculation on one tree of the series inoculated with the Ohio cultures and two days later on a tree inoculated with the Nebraska culture. After 29 days a definite wilt was present in 12 of the 14 trees inoculated. The organism was recovered in 90 per cent of the isolations from inoculated trees showing symptoms. The control trees injured similarly did not produce any disease symptoms.

The progress of the disease varied considerably, the first symptoms appearing on similarly inoculated trees over a period of 18 days. After the first symptoms developed the advance of the disease upward was quite rapid, as shown in Figure 12. The disease often appeared on only one side of the tree as shown in Figure 2 A, and in many instances new branches developed below the point of inoculation as shown in Figure 2B.

Experiment II—Method of Entrance.—An experiment was started in the spring of 1932 to test the possible methods by which infection might occur. The tests were conducted on



A B

FIG. 12.—White-elm seedlings inoculated by inserting mycelium of Cephalosporium into a slit in the trunk. (A) Inoculated tree (left) and control tree (right) 20 days after inoculation showing the first symptoms of the disease. (B) Same trees 10 days later, showing the rapid progress of the disease and the death of all parts above the point of inoculation.

white elm seedlings that were planted in nursery rows in the field. The final readings were made in July, 1933, at which time isolations were made from infected trees. They were divided into eight sets and the methods of inoculation with the results obtained were as follows:

Set 1.—Fifteen trees were sprayed with a suspension of spores and mycelium after the leaves had developed for two weeks. The twigs were injured by needle pricks just preceding the inoculation. Eight of these inoculated trees were covered with moist chambers for 24 hours after inoculation. One of these latter eight trees developed wilt symptoms in 33 days and eventually the lower branches on one side of the tree developed severe die-back. The tree had not been killed, however, at the end of 14 months. One other tree showed severe vascular discoloration but no external symptoms. The other 13 trees remained healthy.

Set 2.—Fifteen trees were sprayed with a spore suspension as in Set 1, but without injury to the twigs. Eight of the trees were covered with moist chambers for 24 hours. All of these trees remained healthy.

Set 3.—Fifteen trees were inoculated by insertion of spores and mycelium in a slit made in the trunk. Two of these trees developed symptoms in 33 and 48 days, and were affected with severe die-back of the tops. They were considerably stunted, although the lower portions of the trees were not affected after 14 months. One other tree developed typical symptoms in 48 days and was dead at the end of three months. The other 12 trees remained healthy.

Set 4.—Fifteen trees were inoculated by hypodermic injections of a spore suspension. Seven of these trees became infected, two of them dying inside of three months and the others showing varying degrees of wilt and die-back with distinct vascular discoloration, although still alive at the end of 14 months.

Set 5.—Ten control trees remained healthy throughout the experiment.

Set 6.—Ten control trees with root injuries as in Set 7, but without inoculum, remained healthy.

Set 7.—Ten trees were inoculated by inserting mycelium and spores into the roots. Five trees developed wilt with vascular discoloration extending throughout the roots and tops. Three of these five trees died within three months. Two additional trees when dug up after 14 months showed severe vascular discoloration throughout the tree, but no external symptoms other than a general stunting were observed on the tops. All of the seven infected trees were stunted and the leaves were smaller and yellowish. The causal organism was isolated from both roots and tops.

Set 8.—Ten trees with injured roots were dipped into a spore suspension before transplanting. Six of these trees were somewhat stunted, with the leaves smaller and yellow but without evidence of die-back or positive wilt. When dug up after 14 months the vascular systems throughout both tops and roots were found to be typically discolored and the causal organism was isolated from both tops and roots.

It is evident from the results of this experiment that the disease may develop from either root or top infection and that root infections result in a general wilt or stunting but without the die-back symptoms so characteristic of top infections.

Experiment III.—The preceding experiment was repeated in the greenhouse during the winter of 1932-33 with the following results:

Set 1.—Five trees were inoculated by the root-incision method. Three of these trees failed to grow and the other two remained healthy.

Set 2.—Five trees were inoculated by dipping the roots in a spore suspension. One tree failed to grow and the other four remained healthy.

Set 3.—Ten trees were atomized with a spore suspension without injury. One of these trees developed wilt and die-back in five weeks and another one in seven weeks. After four months one additional tree was infected and the other seven were healthy.

Set 4.—Ten trees were inoculated similarly to Set 3 but following needle-prick injuries to twigs and leaves. After four months one of these trees showed slight symptoms of wilt with vascular discoloration of the branches. Cephalosporium was isolated from this tree and from the trees in the other sets listed above as infected.

In this experiment fewer trees were infected as the result of root inoculations than in the previous field test. However, the number of trees that developed in Set 1 and Set 2 were too small to make the negative results significant. The percentage of infection resulting from atomizing a spore suspension on the foliage was higher than in the field, as might be expected under the more humid conditions of the greenhouse. These trees were also held in moist chambers for 24 hours after inoculation. Three out of the 10 trees inoculated without injuries developed the disease, while only one of those injured became infected. It is quite probable that accidental

injuries to these trees occurred in handling them and that the pathogene entered through such wounds.

A detailed study of the progress of the disease in one of the trees in Set 3 indicated that infection had occurred in the twig, probably through accidental injuries rather than through the leaves. This tree was cut up eight days after the first symptoms appeared as a yellowing and rolling of a single leaf followed by similar rolling of two more leaves on the next higher twig on the same side of the tree. Cultures were made from the affected leaves and from nine different sections of the branch. Cephalosporium was isolated from all the sections of the twigs but not from the leaves. The organism was present in the vascular system of the branch in an area involving seven side branches but only the upper two twigs or side branches in this infected area had developed symptoms when the tree was cut up.

Experiment IV.—The possibility of natural root infection was tested in the greenhouse in the winter of 1932-33. Fifteen seedlings were planted in soil previously sterilized and inoculated with three-weeks-old cultures of Cephalosporium on Melilotus stems. A similar set was grown in non-sterilized soil to which leaf mold was added from infected trees. None of these trees developed symptoms during the next six months and all isolations from discolored tissue in both roots and tops were negative.

Experiment V.—Another experiment with root inoculations was started in the spring of 1933 with the following results:

Set 1.—Ten trees were inoculated by the root-incision method at time of planting. Severe wilt symptoms developed on one tree in four weeks and on another in five weeks. These two trees were dug up and examined and cultures were made from one of them. The three-foot tree was divided into nine sections, two of them being from the roots. Cephalosporium was recovered from one of the two root sections and from four of the seven top sections, including the section next to the tip of the tree. The organism had progressed very rapidly throughout the tree during the five-week period.

The remaining trees were dug up and examined 14 weeks after inoculation. Two of the trees at this time were normal, two of them had died following wilt symptoms, and the other four trees showed severe brown streaking of the vascular system of both roots and tops with extensive production of suckers. Cephalosporium was isolated from both tops and roots of these trees.

Set 2.—Ten trees were inoculated by dipping roots in a spore suspension at time of planting. At the close of the experiment all of these trees appeared healthy externally but dissection showed that the vascular system of two of them was severely discolored and Cephalosporium was isolated from the discolored portions of both tops and roots.

Chinese Elms.—Chinese elms were tested for susceptibility by inoculating 18 trees with Cephalosporium by hypodermic injections and by stem and root incisions. None of these trees developed symptoms of the disease. When dissected and cultured after four months, it was found that there was a slight discoloration for not more than 2 mm. at the point of inoculation and that the organism had failed to penetrate farther than the injured tissue. Isolations from the tissue around the wounds were uniformly negative.

Summary of Inoculation Tests.—The results of the inoculation tests (Table 1) show that the highest percentages of infection were obtained by introducing the inoculum into the roots or trunks. Much less infection resulted from dipping the roots in a spore suspension when transplanting and still less from atomizing the tops with a spore suspension. No infection resulted from planting trees in inoculated soil in the two tests attempted. No infection was obtained by inoculation of Chinese elms.

TABLE 1.—*Summary of results obtained by inoculation of three-foot white elm seedlings with Cephalosporium* [1]

Method of inoculation	Number of trees	Percentage infected
Inoculum inserted in roots	22	68
Inoculum inserted in trunk	35	54
Hypodermic injection in trunk	15	47
Roots dipped in spore suspension	24	33
Tops atomized with spore suspension [2]	50	12
Soil inoculation with pure culture	15	0
Soil inoculation with infected leaves	15	0

[1] The results of the inoculations were determined by positive symptoms or by isolation of the organism in case the symptoms were questionable.

[2] Similar results were obtained regardless of whether or not the twigs had been injured by pin pricks before atomizing.

EXTENT AND RAPIDITY OF INVASION

The progress of the organism in relation to the development of external and internal symptoms was studied in a number of the inoculated trees. The tree shown in Figure 13 is a good example.

In this tree the inoculation was made above the first branch. The first symptoms appeared 16 days after inoculation. The photograph shown in Figure 13 was taken five weeks later, at which time the tree was cut into two-inch sections. No ex-

FIG. 13.—White-elm seedling 51 days after inoculation at point marked "A". At this time no external symptoms had developed below the point of inoculation but internal discoloration had occurred and the organism was isolated from the area marked "X".

ternal symptoms had developed at this time below the point of inoculation and vascular discoloration was present for only a short distance. When sectioned and cultured it was found that the causal organism extended down the tree some five inches and out into the branch an additional five inches. Cephalosporium was recovered from sections above the point of inoculation, all the upper portion of the tree being invaded. The organism progressed much more rapidly in an upward than in a downward direction in this tree.

A good example of the annual progress of the disease in a naturally infected tree was found on the Agricultural College campus. This white elm was 15 inches in diameter. The first symptoms of the disease were noted in the summer of 1931, although the disease had evidently started the previous year, as shown by die-back of the upper branches (Fig. 14 A), from which the organism was isolated. These branches produced small, yellow leaves in the spring of 1931 and were dead by midsummer. The next symptoms appeared in the next lower branch and also on the tips of three other branches. Cephalosporium was isolated from the discolored tissue of these affected twigs. By 1932 all of the top was affected, with the exception of a portion of one of the main branches (Fig. 14 B). The branches that showed their first symptoms in 1932 started out with normal leaf production, but many of the leaves started to yellow, roll, and wilt before reaching normal size, in a manner similar to that of trees in the root-inoculation tests. Cephalosporium was again isolated from the affected branches. This tree was cut down in the late fall of 1932 and cut into sections for further study. Cephalosporium was isolated from all portions of the tree that had developed symptoms, from the main trunk about three feet above the ground level, and from 10 segments taken at two-foot inter-

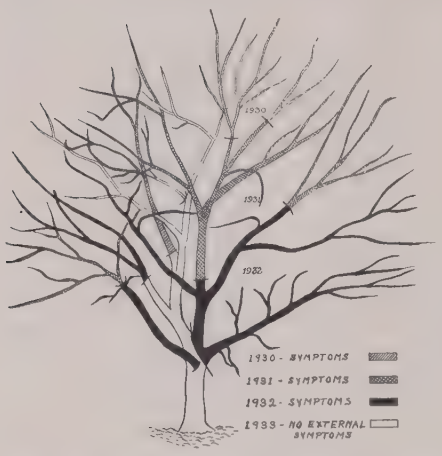


FIG. 14A.—Sketch of a naturally infected tree showing the annual progress of the disease.

vals from the base to the tip of the branch that did not show any external symptoms during the summer of 1932. The internal discoloration extended throughout the tree. The heart wood of the trunk was discolored down to the ground line and some discoloration of the roots was found, but no cultures of Cephalosporium could be obtained from a segment of trunk just above the ground line or from the roots, although 63 tissue cultures were made from these portions.

It was evident from the history of this tree that the disease is definitely capable of causing the death of good-sized trees in two or three years. The yearly progress as shown in Figure 14 A also indicates that while the organism did advance down the branches and trunk, infection also had occurred in new branches each year by penetration from the outside rather than by the gradual internal invasion of the tree. The inoculation experiments have shown that the disease progresses rapidly in an upward direction and as a result the length of time necessary for the death of a tree will depend to a great extent upon the point or points of infection.

DISCUSSION

While there are many phases of this disease that need further investigation, evidence has been obtained regarding the causal organism and the development of the disease, both by inoculation experiments and by the study of naturally infected trees.

It is evident that this species of Cephalosporium is capable of infecting the white elm and of killing large trees in a relatively short time. The disease is of common occurrence in Nebraska and also in a number of other states, as reported by May and Liming.

The rapidity of the development of the disease depends to a great extent upon the point of infection. When infection occurs through the branches the disease develops as a wilt

and die-back of the individual branches. If the lower branches are first affected the penetration of the organism into the trunk may result in a rapid wilting of the whole tree without the die-back of individual branches in the upper portions. Likewise if the trunk becomes infected from the roots the entire tree may wilt and die in a short time.

FIG. 14B.—Photograph taken in 1932 of the tree shown in Fig. 14A.

There have been a few instances, both in naturally infected trees and in those used in the inoculation tests, where trees have been infected and the organism found throughout the vascular system but where the external symptoms have been very slight and the trees were still surviving after 14 months. The organism has been consistently isolated from one young tree about six inches in diameter for the past three summers and while the tree has been pruned back a number of times it does not seem to be suffering greatly. Such trees while tolerant of the disease, may serve as sources of infection for other more susceptible trees.

The fact that the organism is confined to the vascular system and that it has not yet been found fruiting on the surface of diseased wood limits the probability of aerial spread of the disease. While no specific insects have yet been associated with this trouble, it is possible that they play an important part as agents of both dissemination and inoculation. Such insect injuries as shown in Figure 4 A could easily be the means of initiating the disease. Infected wood thus punctured could serve as a source of inoculum. The fact that spore suspensions sprayed on trees resulted in infection re-

gardless of whether the leaves and twigs were injured would also indicate that deep or severe injuries of young twigs are not necessary and that slight injuries such as occur in handling seedlings or abrasions due to the rubbing of twigs in the wind might serve as openings for infection.

While infection has been obtained by inoculating roots, there is still a need for further investigation on this phase of the disease as a natural means of spread. The petioles of leaves have been found to carry the organism and it was thought that such infected leaves dropping to the ground might serve as sources of root infection through the soil. The few tests with such leaves as inoculum and also the pure-culture inoculations of the soil failed to produce the disease. There is also the possibility of replants becoming infected from the old roots but so far the organism has not been recovered from the roots of naturally infected trees.

There have been a number of instances of this disease taking an entire row of trees in street plantings, in a relatively short time. The first observation made of the disease in this state was an instance of this type. When first observed three or four trees in a row were definitely infected and almost completely defoliated by midsummer. The upper branches of the adjoining trees showed slight symptoms and the following year the disease was present in some 15 to 20 adjoining trees, at which time they were all removed.

Tests are needed to determine the relative susceptibility of other species of elm. The Chinese elm has proved resistant in the few tests conducted but no other species have been tested. The use of resistant species for planting in affected areas, particularly as replants, is an important phase of the problem. There has been no evidence in this investigation that the vigor of the tree has any great influence upon infection or development of the disease.

The only method of control available at present is the careful inspection and removal of infected trees from nurseries, the removal of seriously infected trees from ornamental plantings, and the pruning out of affected portions of trees slightly infected. The pruning of diseased branches is apt to prove disappointing in many instances because the success of this measure depends upon removal very soon after infection, particularly if the point of infection is close to large limbs or to the trunk, and the practice is further made difficult by the fact that the organism develops internally considerably in advance of the external symptoms. The cut must be made several inches beyond the most advanced point of internal discoloration.